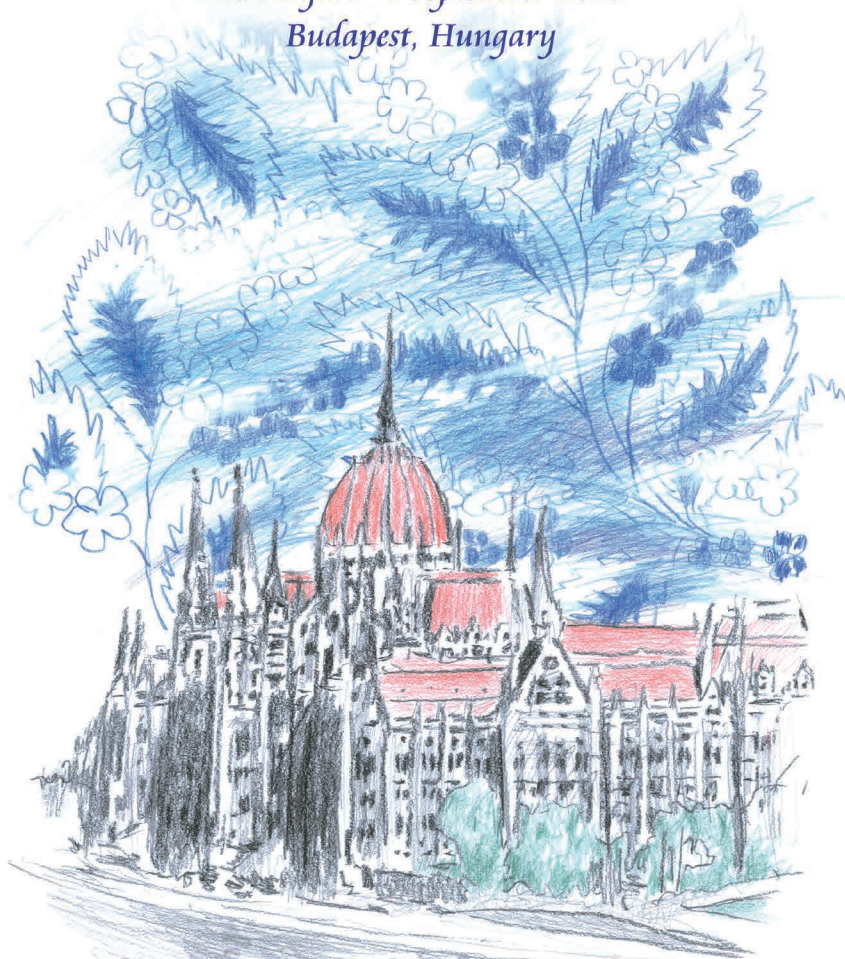


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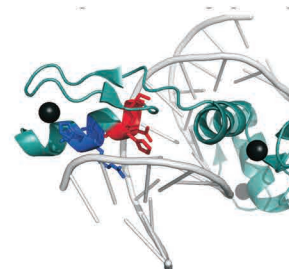
Kovács

TURNING DESIGNED ZINC FINGER PROTEINS INTO ARTIFICIAL NUCLEASES

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Zinc finger proteins are modular specific DNA binders that can be designed to target any selected DNA nucleotide sequence.¹ As such, they can control the function of genes, but they also can be turned into specific DNA manipulating agents. Previously it was reported that Ni(II)-ions can induce peptide bond hydrolysis that occurs at the N-terminal side of Ser or Thr residues in peptides or proteins, bearing X-(Ser/Thr)-X-His-X sequences (X being any amino acid except of Pro before or after Ser/Thr).²⁻⁴ By means of a hydrolytic reaction of this kind we can specifically cleave zinc finger proteins using nickel(II) ions. The cleavage results in a square planar nickel(II) complex at the N-terminus of the C-terminal cleavage product. The latter complex is known as ATCUN motif, which is able to cleave DNA.⁵ Based on these facts, the combination of the hydrolytic experiment with zinc finger design will, according to our expectations, lead to the development of in situ prepared specific artificial nucleases.



For this purpose, we optimized the conditions of the specific cleavage of the zinc finger proteins both in the absence and presence of DNA. We elaborated a precise protocol to study the specific cleavage of the P-1MEY# zinc finger protein – which was redesigned to contain only one cleavage site for nickel(II) ions, as the published 1MEY zinc finger array⁶ included two inherent sequences sensitive to nickel(II)-induced hydrolysis. Combined electrophoretic, HPLC, CD and MS measurements were applied to monitor the reaction, to check the metal ion binding status, and to determine the solution structure of the complexes formed.

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